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Subunit communication in the tryptophan synthase $\alpha_2\beta_2$ complex

Effects of β subunit ligands on proteolytic cleavage of a flexible loop in the α subunit

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To probe the structural basis for ligand-mediated communication between the α and β subunits in the tryptophan synthase $\alpha_2\beta_2$ complex, we have determined the effects of ligands of the α and β subunits on proteolysis of a flexible loop in the α subunit. We find that addition of a ligand of the β subunit (L-serine, D-tryptophan, or L-tryptophan) in combination with a ligand of the α subunit (α-glycerol 3-phosphate) almost completely prevents the tryptic cleavage of the α subunit loop. Thus, the binding of a ligand to the β-site affects the conformation of the α subunit 25–30 Å distant.

Tryptophan synthase; Subunit communication; Allosteric mechanism; Protein loop; Proteolysis; Ligand binding

1. INTRODUCTION

An important problem in the elucidation of the allosteric mechanism is the structural basis for ligand-mediated communication between topologically distinct binding sites. An ideal system for investigating this problem is the bacterial tryptophan synthase $\alpha_2\beta_2$ complex (EC 4.2.1.20) that catalyzes the final reactions in the biosynthesis of L-tryptophan [1–3]. Crystallographic studies show that the α and β active sites are 25–30 Å apart and are connected by a tunnel [4]. Since ligands that bind at one active site influence the properties of the other site, the heterologous sites communicate reciprocally over a distance of 25–30 Å [5–7]. These allosteric interactions result from ligand-induced conformational changes that are transmitted from one protomer to the other.

Trypsin cleaves the tryptophan synthase $\alpha_2\beta_2$ complex at Arg-188 in the α subunit and produces two fragments termed α-1 and α-2 [8,9]. Arg-188 is located in a long, disordered loop in the α subunit (residues 179–192) that can not be seen in the crystal structure of the $\alpha_2\beta_2$ complex from *Salmonella typhimurium* [4,10]. Our finding that addition of a ligand of the α subunit decreases the rate of cleavage by tripsin suggest that ligand binding alters the conformation and flexibility of te loop [11]. An allosteric role for the α subunit loop is supported by the observation that the native $\alpha_2\beta_2$ complex is strongly inhibited by a ligand of the α subunit whereas the 'nicked' $\alpha_2\beta_2$ complex is desensitized to this inhibition [11].

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2. MATERIALS AND METHODS

2.1. *Enzymes, assays, and proteolysis*

Tryptophan synthase $\alpha_2\beta_2$ complex from *S. typhimurium* was purified as described [12]. Solutions of the $\alpha_2\beta_2$ complex (1.6 mg/ml in 50 mM sodium *N,N*-bis(2-hydroxyethyl)glycine buffer containing 1 mM EDTA at pH 7.8) were treated at 22°C with 1 μg/ml TPCK-trypsin (Cooper Biomedical) in the presence or absence of ligands; reactions were stopped by addition of trypsin inhibitor [11]. Aliquots (5–10 μl) were assayed for activity in the synthesis of L-tryptophan from indole and L-serine in the presence or absence of 80 mM DL-α-glycerol 3-phosphate (Sigma) [13].

2.2. *Gel electrophoresis and densitometry*

Sodium dodecyl sulfate gel electrophoresis of proteins and staining with Coomassie blue R-350 utilized a Phast System (Pharmacia LKB Biotechnology) [11]. The Hoefer GS-360 Data System and GS-300 scanning densitometer were used to scan gels stained with Coomassie blue R-250. Areas of peaks (α subunit and α-1 fragment) were obtained by Gausian integration. The fractional cleavage is defined as the (area of α-1 fragment)/ (area of α-1 fragment + area of α subunit). This calculation disregards the very small peak due to a second product of proteolysis, the α-2 fragment [8,9].

3. RESULTS

The present study asks whether ligands bound to the β-site communicate allosteric effects to the α subunit loop. We have determined the effects of ligands of the α and β subunits on the rate of tryptic cleavage of the α subunit in the $\alpha_2\beta_2$ complex (Fig. 1A and B) and on the ratio of activity in the presence of α-glycerol 3-phosphate to the activity in the absence of α-glycerol 3-

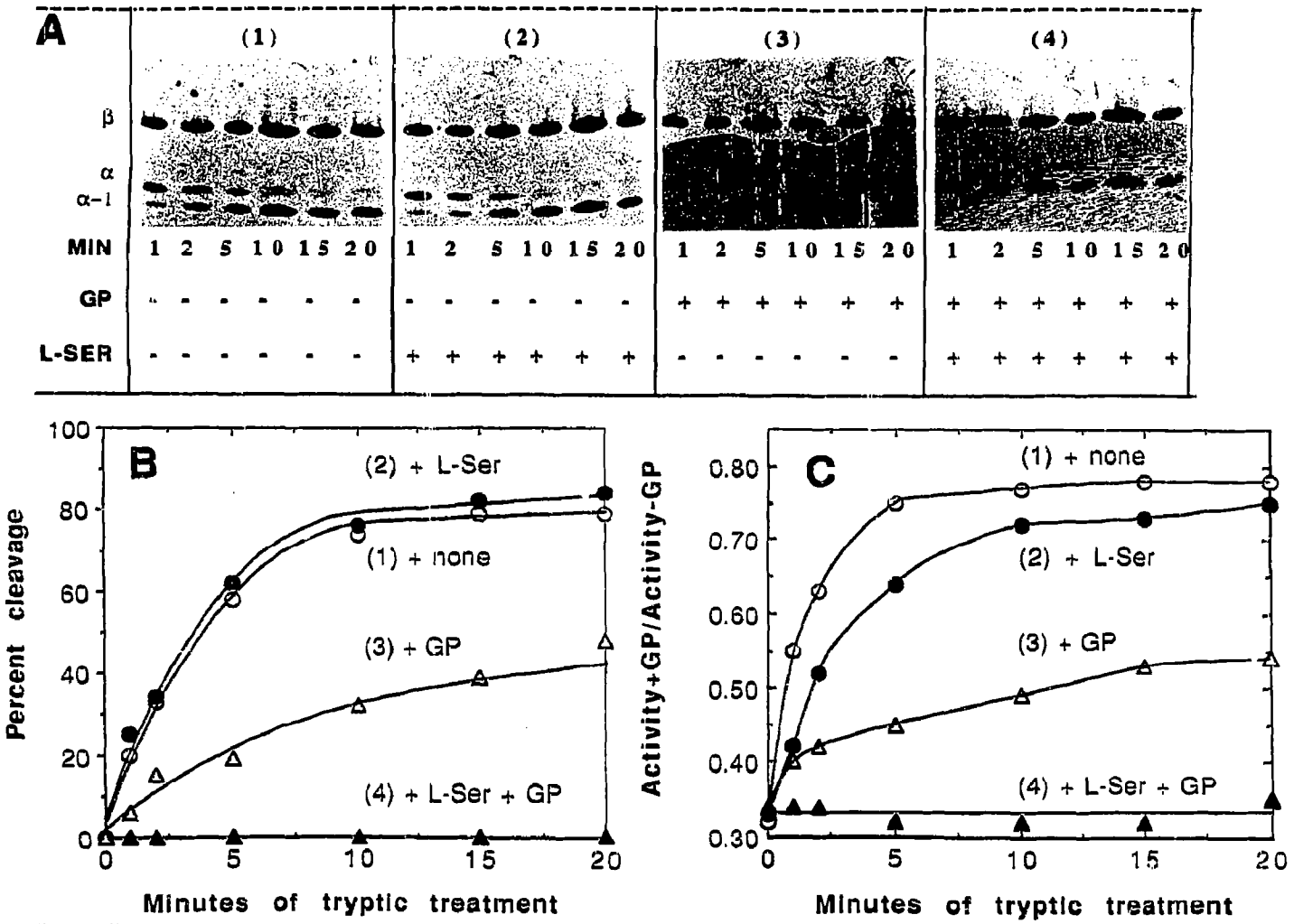

Fig. 1. Effect of ligands on the time course of proteolysis of the tryptophan synthase $\alpha_2\beta_2$ complex as determined by sodium dodecyl sulfate gel electrophoresis (A), by densitometric analysis of the gels (B), and by determination of the relative activity (Activity + GP/Activity – GP) (C).

phosphate (Fig. 1C). An increase in this ratio reflects the activation or densensitization ot inhibition that results from cleavage [11]. We find that L-serine alone has no effect on the rate of tryptic cleavage or of activation. In contrast, the α subunit ligand, α-glycerol 3-phosphate, decreases the rate of cleavage and of activation, as previously reported [11]. An important new observation is that addition of L-serine in combination with α-glycerol 3-phosphate almost completely prevents cleavage and activation. The rates of activation of the $\alpha_2\beta_2$ complex during proteolysis (Fig. 1C, curves 1–4) are similar to the rates of proteolysis (Fig. 1B, curves 1–4).

Table I shows the protective effects of ligands of the α and β subunits. L-Serine and low concentrations of L-tryptophan and D-tryptophan, which bind to the enzyme, give strong protection from cleavage and from activation in the presence of α-glycerol 3-phosphate but have much smaller effects in the absence of α-glycerol 3-phosphate. The nonsubstrate amino amino acids, L-alanine and D-alanine, have no effect in the presence or absence of α-glycerol 3-phosphate. In the presence of α-glycerol 3-phosphate, a high concentration of D-serine has a small effect whereas a low concentration has no effect.

4. DISCUSSION

The flexible loop in the α subunit plays important roles in ligand binding and in communicating the effects of ligand binding from the α subunit to the β subunit [11,14]. Our new finding that a ligand at the β site stabilizes the α subunit loop in the presence of a ligand of the α subunit is evidence that communication between the α subunit loop and the β site is reciprocal. Reciprocal communication between the α and β sites results from the transmission of ligand- induced conformational changes between the active sites [5–7]. Our finding that L-serine alone does not prevent loop cleavage shows that the effects of L-serine on proteolysis

Table I

Effects of ligands of the α and β subunits on proteolytic cleavage of a flexible α subunit loop in the tryptophan synthase $\alpha_2\beta_2$ complex[a]

β subunit ligand		α subunit ligand	Protection from cleavage[b] (%)	Protection from activation[c] (%)
Addition	Conc.	Addition		
None		GP	62	63
L-Serine	50 mM	None	0	11
L-Serine	50 mM	GP	92	100
L-Tryptophan	1 mM	None	15	0
L-Tryptophan	1 mM	GP	70	100
D-Tryptophan	1 mM	None	16	23
D-Tryptophan	1 mM	GP	75	100
D-Serine	1 mM	None	0	0
D-Serine	10 mM	None	0	0
D-Serine	50 mM	None	0	0
D-Serine	1 mM	GP	46	69
D-Serine	10 mM	GP	72	83
D-Serine	50 mM	GP	86	80
D-Alanine	50 mM	None	0	0
D-Alanine	50 mM	GP	60	62
L-Alanine	50 mM	None	0	0
L-Alanine	50 mM	GP	57	66

[a] The $\alpha_2\beta_2$ complex was treated with trypsin for 10 min in the presence or absence of the indicated β subunit ligand and α subunit ligand (80 mM DL-α-glycerol 3-phosphate; GP) as described in Fig. 1.

[b] % protection from cleavage = 100 × {(%α + ligands) − (%α − ligands)}/{100 − (%α − ligands)}, where %α = % α subunit remaining after 10 min proteolysis as determined by densitometry. An example of the calculation for the first experiment with GP alone, % protection from cleavage = 100 × (69 − 26)/(100 − 26) = 58%.

[c] % protection from activation = 100 × {Tr(−L) − Tr(+L)}/{Tr(−L) − UnTr} is calculated from the relative activity (Activity + GP/ Activity − GP) of enzyme treated in the absence of ligands {Tr(−L)}, in the presence of ligands {Tr(+L)}, or untreated {UnTr}. An example of the calculation is given for the first experiment with GP alone: % protection from activation = 100 × (0.76 − 0.49)/(0.76 − 0.33) = 63%.

differ from the effects of L-serine on subunit dissociation [14–17] and on heat denaturation (Ruvinov and Miles, unpublished results). L-Serine alone strongly protects the $\alpha_2\beta_2$ complex from dissociation and from heat denaturation; the addition of L-serine and α- glycerol 3-phosphate in combination results in stronger protection. We conclude that our studies reveal a specific effect related to the loop in the liganded α subunit. The loop may undergo a conformational change upon binding α-glycerol 3-phosphate that is further stabilized by an L-serine-induced conformational change in the β subunit. This hypothesis is supported by our finding that ligands do not protect and $\alpha_2\beta_2$ complex with a mutation in the loop (T183A) [17] but do protect several other mutant $\alpha_2\beta_2$ complexes (Ruvinov and Miles, unpublished results). We anticipate that future crystallographic studies of the $\alpha_2\beta_2$ complex with ligands bound at both α and β sites will disclose changes in the α subunit loop that result from ligand-mediated subunit communication.

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